ANTIBODY TO HEPATITIS B SURFACE ANTIGEN
(Mouse Monoclonal)

Genetic Systems™ HBsAg EIA 2.0

Enzyme Immunoassay (EIA) for Detection of Hepatitis B Surface Antigen (HBsAg) in Human Serum, Plasma, and Cadaveric Serum Specimens

For In Vitro Diagnostic Use

32561 • 480 Tests
32562 • 960 Tests
32563 • 4800 Tests

Manufactured for Sanofi Diagnostics Pasteur, Inc. by
Genetic Systems Corporation
U.S. License No. 978

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NAME AND INTENDED USE

Genetic Systems™ HBsAg EIA 2.0 is a qualitative enzyme immunoassay for the detection of Hepatitis B Surface Antigen (HBsAg) in human serum and plasma, and also in cadaveric serum specimens. The HBsAg EIA 2.0 is intended to be used for screening blood and blood products intended for transfusion or for further manufacture into plasma products.

SUMMARY AND EXPLANATION OF THE TEST

Hepatitis B virus (HBV) is a major public health problem worldwide, with significant transmission of the virus occurring through the use of contaminated donor blood and plasma. Also of concern is the transmission of HBV and other infectious diseases through tissue transplantation.[1] Because the presence of circulating Hepatitis B Surface Antigen (HBsAg) closely follows the course of infection, screening for HBsAg is used to detect potentially infectious blood and plasma.[2] Enzyme immunoassays to detect HBsAg have replaced relatively insensitive gel diffusion methods and have been reported to have equivalent sensitivity to radioimmunoassay methods.[3] The application of monoclonal antibodies for the detection of HBsAg has previously been reported.[4,5] The Genetic Systems™ HBsAg EIA 2.0 is a third generation enzyme immunoassay, which uses mouse monoclonal antibodies to detect HBsAg in human serum, plasma, or cadaveric serum specimens.

Specimens that are nonreactive when tested with the Genetic Systems™ HBsAg EIA 2.0 are considered negative for HBsAg and need not be tested further. Reactive specimens should be retested, in duplicate, using the Genetic Systems™ HBsAg EIA 2.0 to determine whether they are repeatedly reactive. A repeatedly reactive specimen should be confirmed by a licensed neutralization procedure utilizing human anti-HBs (HBsAg Confirmatory Assay). If the HBsAg in the specimen can be neutralized by the confirmatory procedure, the specimen is considered positive for HBsAg and need not be tested further.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

Wells of the microwell strip plates are coated with mouse monoclonal antibody to HBsAg (anti-HBs). Serum or plasma and appropriate controls are added to the wells, and incubated with the bound antibody. If HBsAg is present, it will bind to the antibody and not be removed by washing. The strips are washed to remove any unbound material. Washing is followed by the addition of Conjugate Solution (peroxidase-conjugated mouse monoclonal antibodies directed against HBsAg). The Conjugate Solution will bind to the antibody-HBsAg complex, if present. Unbound conjugate is removed by a wash step. Next, Working Chromogen Solution is added to the plate and allowed to incubate. A blue or blue-green color develops in proportion to the amount of HBsAg present in the sample. The enzyme reaction is stopped by the addition of acid, which changes the blue-green color to yellow. The optical absorbance of specimens and controls is determined with a spectrophotometer set at 450 nm wavelength.

REAGENTS

Genetic Systems™ HBsAg EIA 2.0 Product Description

Product No: 32561 (480 Tests), 32562 (960 Tests), 32563 (4800 Tests)

Component	Contents	Preparation
R3• HBsAg Conjugate Concentrate 1 or 5 vial(s) (1.2 ml)	•Anti-HBsAg (mouse monoclonal): horseradish peroxidase conjugate •Buffer with protein stabilizers •0.005% Gentamicin Sulfate •0.5% Proclin 300™	Dilute in HBsAg Conjugate Diluent as described.
R1• Anti-HBsAg Microwell Strip Plates, 5, 10 or 50	•Microwell strips in holder, coated with antibody to HBsAg (mouse monoclonal) •Sodium Azide •Proclin 150™	Use as supplied. Return unused strips to the pouch. Do not remove desiccant.
C0• HBsAg Negative Control (Human) 1 or 5 vial(s) (12 ml)	•Normal Human Serum •Nonreactive for HBsAg, Anti-HBsAg •Nonreactive for Antibody to HIV, HTLV-I/II, HCV •0.005% Gentamicin Sulfate •0.5% Proclin 300™	Ready to use as supplied.
C1• HBsAg Positive Control (Human) 1 or 5 vial(s) (8 ml)	•Normal Human Serum containing HBsAg •Nonreactive for Anti-HBsAg •Nonreactive for Antibody to HIV, HTLV-I/II, HCV •0.005% Gentamicin Sulfate •0.5% Proclin 300™	Ready to use as supplied.
C2• HBsAg Low Positive Control (Human) 1 or 5 vial(s) (8 ml)	•Normal Human Serum containing HBsAg •Nonreactive for Anti-HBsAg •Nonreactive for Antibody to HIV, HTLV-I/II, HCV •0.005% Gentamicin Sulfate •0.5% Proclin 300™	Ready to use as supplied.
R4• HBsAg Conjugate Diluent 1 or 5 bottle(s) (120ml)	•Buffer with protein stabilizers •0.005% Gentamicin Sulfate •0.5% Proclin 300™	Ready to use as described under Working Conjugate Solution.
R5• EIA Wash Solution Concentrate (30X) 2, 3 or 15 bottle(s) (120 ml)	•Sodium Chloride •Tween 20	Dilute to working dilution with deionized water.
R6• EIA Chromogen Reagent 1 or 5 vial(s) (1.5 ml)	•Tetramethylbenzidine (TMB)* •Dimethylsulfoxide (DMSO)	Dilute with EIA Chromogen Diluent as described.
R7• EIA Chromogen Diluent 1 or 5 bottle(s) (120ml)	•Hydrogen Peroxide •Citric Acid •Dimethylsulfoxide (DMSO)	Ready to use as supplied.
R8• EIA Stopping Reagent 1 or 5 bottle(s) (120ml)	•1N H_2SO_4	Ready to use as supplied.
• Plate Sealers	•Clear plastic sealers	Ready to use as supplied.

*NOTE: Tetramethylbenzidine is a non-carcinogenic and non-mutagenic chromogen for peroxidase.[6,7]

Store the kit at 2–8°C. Bring all reagents except Conjugate Concentrate to room temperature (15-30°C) before use. Return to 2-8°C immediately after use. Store all unused strips/plates with desiccant at 2-8°C.

WARNINGS FOR USERS
For *In Vitro* Diagnostic Use

1. The Genetic Systems™ HBsAg EIA 2.0 contains human blood components. No known test method can offer complete assurance that products derived from human blood will not transmit infection. Therefore, all human blood derivatives should be handled as though they contain an infectious agent. Handle these reagents and human specimens using the precautions recommended for bloodborne pathogens, as defined by OSHA regulations.

2. Do not pipette by mouth.

3. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.

4. Wear protective clothing and disposable gloves while handling the kit reagents. Wash hands thoroughly after performing the test.

5. Handle EIA Chromogen Reagent and Chromogen Diluent with care, since DMSO is readily absorbed through the skin.

6. The EIA Stopping Reagent is a strong acid. Wipe up spills immediately. Flush the area of the spill with water. If the Stopping Reagent contacts the skin or eyes, flush with copious amounts of water and seek medical attention.

7. BIOLOGICAL SPILLS: Spills not containing acid should be wiped thoroughly with an effective disinfectant. Disinfectants that are known to inactivate the virus include (but are not limited to) a solution of 10% bleach (0.5% solution of sodium hypochlorite), 70% ethanol, or 0.5% Wescodyne™.[8-10] Spills containing acid should be wiped dry. The area of the spill should be wiped with one of the chemical disinfectants. Materials used to wipe up spills should be disposed of as biohazardous waste. NOTE: DO NOT PLACE SOLUTIONS CONTAINING BLEACH IN THE AUTOCLAVE.

8. Dispose of all specimens and materials used to perform the test as though they contain an infectious agent. Disposal should comply with all applicable waste disposal requirements.[8-10]

PRECAUTIONS FOR USERS

1. Do not use the kit beyond the stated expiration date.

2. The only reagents that may be used with different lots of the HBsAg EIA 2.0 are the EIA Chromogen Reagent, EIA Chromogen Diluent, EIA Wash Solution Concentrate, and EIA Stopping Reagent. Do not mix any other reagents from different lots.

3. Do not use the EIA Chromogen Diluent for the EIA Buffered Substrate in other Genetic Systems Tests.

4. Exercise care when opening vials and removing aliquots to avoid microbial contamination of the reagents.

5. Use a clean, disposable container for the conjugate. Exposure of the conjugate to sodium azide will result in its inactivation.

6. Avoid exposing EIA Chromogen Reagent or Working Chromogen Solution to strong light during storage or incubation. Do not allow the Working Chromogen Solution to come into contact with an oxidizing agent.

7. Avoid contact of the EIA Stopping Reagent with any oxidizing agent. Do not allow EIA Stopping Reagent to come into contact with metals.

8. Use clean, polypropylene containers to prepare and store the Working Chromogen Solution. If glassware must be used, pre-rinse thoroughly with 1N sulfuric or hydrochloric acid followed by at least three washes of deionized water. Be sure that no acid residue remains on the glassware.

9. Bring all reagents except Conjugate Concentrate to room temperature before use.

10. For the manual pipetting of controls and specimens, use individual pipette tips to eliminate carryover of samples.

11. Handle the Negative and Positive Controls in the same manner as patient specimens.

12. If a specimen or reagent is inadvertently not added to a well, the assay results will read negative.

13. Inadequate adherence to package insert instructions may result in erroneous results.

14. Use only adequately calibrated equipment with this assay.

15. Use of dedicated equipment is recommended if equipment performance validations have not precluded the possibility of cross-contamination.

16. The Genetic Systems™ HBsAg EIA 2.0 performance is highly dependent upon incubation times and temperatures. Temperatures outside of the validated ranges may result in invalid assays. Incubation temperatures should be carefully monitored using calibrated thermometers, or equivalent.

17. Components of this kit meet FDA potency requirements.

REAGENT PREPARATION AND STORAGE

Working Conjugate Solution

Bring Conjugate Diluent to room temperature. Invert Diluent and Conjugate Concentrate to mix before using. Prepare a 1:101 dilution for each strip to be tested by mixing 10µl of Conjugate Concentrate to 1 ml of Conjugate Diluent in a clean container. Note Concentrate lot number, date and time of preparation, and date and time of expiration (8 hours from preparation) on container. Mix Working Solution when combined and again just prior to use. Working Solution is stable for 8 hours.

Return Conjugate Concentrate to the refrigerator immediately after use. To avoid contamination of Conjugate, wear clean gloves and do not touch tips of pipettes. Store Working Conjugate Solution at room temperature until use.

Prepare only the amount of reagent to be used within 8 hours, ensuring that the volume of diluted reagent will be adequate for the entire run. Use the following table as a guide:

Preparation of Working Conjugate Solution by Strip

Number of Strips to be used	1	2	3	4	5	6	7	8	9	10	11	12*
Amount of Conjugate Concentrate (µl)	10	20	30	40	50	60	70	80	90	100	110	120
Amount of Conjugate Diluent (ml)	1	2	3	4	5	6	7	8	9	10	11	12

* Complete Plate

Preparation of Working Conjugate Solution by Plate

Number of Complete Plates to be used	1	2	3	4	5	6	7	8	9	10
Amount of Conjugate Concentrate (µl)	120	240	360	480	600	720	840	960	1080	1200
Amount of Conjugate Diluent (ml)	12	24	36	48	60	72	84	96	108	120

Working Chromogen Solution

Bring Chromogen Reagent and Chromogen Diluent to room temperature. Invert the Chromogen Reagent and Chromogen Diluent to mix before using. Prepare a 1:101 dilution for each strip to be tested by mixing 10µl of Chromogen Reagent to 1 ml of Chromogen Diluent in a clean, polypropylene container. Note Chromogen Reagent lot number, date and time of preparation, and date and time of expiration (8 hours from preparation) on container. Mix Working Solution gently when combined and again just prior to use. Working Chromogen Solution should be kept in the dark at room temperature and used within 8 hours.

Chromogen Reagent may be in crystalline form at refrigerator temperature and should be allowed to liquefy to room temperature prior to use. If solution remains crystalline at room temperature, do not use. Chromogen Reagent should be colorless to slightly yellow. Any other color indicates that the reagent is contaminated. Do not use this reagent and contact Genetic Systems/Sanofi Technical Services at 1-800-666-2111.

The Working Chromogen Solution should be colorless. A distinct blue color indicates that the reagent is contaminated. Discard the Working Chromogen Solution and prepare fresh reagent in a clean container.

Prepare only the amount of the reagent to be used within 8 hours, ensuring that the volume of diluted reagent will be adequate for the entire run. Extra Chromogen Reagent is provided. Use the following table as a guide:

Preparation of Working Chromogen Solution by Strip

Number of Strips to be used	1	2	3	4	5	6	7	8	9	10	11	12*
Amount of Chromogen Reagent (µl)	10	20	30	40	50	60	70	80	90	100	110	120
Amount of Chromogen Diluent (ml)	1	2	3	4	5	6	7	8	9	10	11	12

* Complete Plate

Preparation of Working Chromogen Solution by Plate

Number of Complete Plates to be used	1	2	3	4	5	6	7	8	9	10
Amount of Chromogen Reagent (µl)	120	240	360	480	600	720	840	960	1080	1200
Amount of Chromogen Diluent (ml)	12	24	36	48	60	72	84	96	108	120

Wash Solution

Prepare Wash Solution by adding one part EIA Wash Solution Concentrate (30X) to 29 parts of deionized water (e.g., 120ml of Wash Solution Concentrate to 3480 ml of deionized water). The diluted Wash Solution can be stored at room temperature for up to four weeks in a plastic container. Note the lot number, date prepared, and expiration date on the container. Discard if no suds are evident in the Wash Solution. Prepare a sufficient quantity of Wash Solution to complete a full run.

SPECIMEN COLLECTION, PREPARATION, AND STORAGE

Serum, plasma, or cadaveric serum specimens may be used. The following anticoagulants have all been evaluated and found to be acceptable: EDTA, heparin, sodium citrate, CPDA-1, and ACD. Samples which are collected into anticoagulant tubes should be filled as labeling indicates to avoid improper dilution. Specimens with observable particulate matter should be clarified by centrifugation prior to testing. No clinically significant effect has been detected in assay results of serum or plasma samples with increased levels of protein, lipids, bilirubin, or hemolysis, or after heat inactivation of patient samples. Cadaveric serum samples with increased levels of hemolysis have been tested, and no clinically significant effect has been detected in assay results. Note: Cadaveric serum samples with increased levels of protein, lipids, bilirubin, or microbiological contaminants have not been available to evaluate with this assay.

Serum, plasma, or cadaveric serum specimens may be stored at 2-8°C for up to seven days. Samples should not be used if they have incurred more than 5 freeze/thaw cycles. Mix samples thoroughly after thawing. NOTE: Cadaveric specimens that are weakly reactive may become nonreactive after freeze/thaw cycles.

If specimens are to be shipped, they should be packed in compliance with Federal Regulations covering the transportation of etiologic agents. Studies have demonstrated that specimens may be shipped refrigerated (2-8°C) or at ambient temperatures for up to 7 days. For shipments that are in transit for more than 7 days, specimens should be kept frozen (-20°C or lower). Refrigerate samples at 2-8°C at receipt, or freeze for longer storage.

This kit is not licensed for use with specimens other than serum, plasma, or cadaveric serum specimens. This kit is not intended for use on saliva/oral fluids or urine samples.

HBsAg EIA 2.0 PROCEDURE

Materials Provided

See Reagents Section on page 3.

Materials Required but not Provided

1. Precision pipettes to deliver volumes from 10 µl to 200 µl, 1 ml, 5 ml, and 10 ml (accurate within ± 5%). A multichannel pipettor capable of delivering 100µl or 200µl is optional.

2. Pipette tips.

3. Dry-heat static or shaker incubator capable of maintaining 37±1°C. If a shaker incubator is used, it should have the following specifications:

   Amplitude: 0.75 to 3.00 mm
   Frequency: 500 to 2300 R.P.M.

4. Genetic Systems microwell plate or strip washer, or an equivalent. The washer must be capable of dispensing 400µl per well, cycling 5 times, and soaking for 30-60 seconds between each wash.

5. Genetic Systems microwell plate or strip reader or an equivalent. The spectrophotometer should have the following specifications at wavelength 450 nm:

   Bandwidth: 10 nm HBW (Half Band Width)
   Absorbance Range: 0 to 2 AU (Absorbance Units)
   Repeatability: ± (0.5% + 0.005) AU
   Linearity or Accuracy: 1% from 0 to 2.0 AU

   The instrument should contain a reference filter for reading at 615 to 630 nm. An instrument without a reference filter can be used; however, areas in the bottoms of the wells that are opaque, scratched, or irregular may cause absorbance readings that are falsely elevated.

6. Household bleach (5% to 8% sodium hypochlorite) which may be diluted to a minimum concentration of 10% bleach (or 0.5% sodium hypochlorite). Alternative disinfectants include: 70% ethanol or 0.5% Wescodyne™ (West Chemical Products, Inc.).

7. Paper towels or absorbent pads for blotting.

8. Labeled null strips, for testing partial plates.

9. Clean, polypropylene containers for preparation of Chromogen and Conjugate Working Solutions, 15 or 50 ml.

10. Deionized water.

11. Gloves.

12. Laboratory timer.

13. EIA reagent reservoirs (optional).

Preliminary Statements

1. The expected run time for this procedure is approximately 3-4 hours from initiation of the first incubation step. Each run of this assay must proceed to completion without interruption after it has been started.

2. Positive and Negative Controls must be run on each plate. The cutoff for patient samples is determined by the controls on each individual plate.

3. The number of controls to be included in each run of this assay are two Positive Controls, two Low Positive Controls, and three Negative Controls.

4. Do not splash controls, specimens, or reagents between microwells of the plate.

5. Cover plates for each incubation step using plate sealers provided or other appropriate means to minimize evaporation.

6. Avoid exposure of the plates to light during the final incubation step (following the addition of the Working Chromogen Solution).

7. Adhere to the recommended time constraints for the use of the Working Chromogen Solution (8 hours), Working Conjugate Solution (8 hours), and Wash Solution (4 weeks).

8. Avoid the formation of air bubbles in each microwell.

There are two procedures for the detection of HBsAg in serum or plasma, procedures A and B. **For the detection of HBsAg in cadaveric serum specimens, only procedure A can be used.** The two procedures for the detection of HBsAg are described below:

Procedure	Specimen incubation	Conjugate incubation	Color development
A	dry heat, 36-38°C, static incubation, 60 min.	dry heat, 36-38°C, static incubation, 60 min.	15 to 30°C; 30 min. in the dark.
B	shaker incubation, 36-38°C, 60 min.	shaker incubation, 36-38°C, 60 min.	15 to 30°C; 30 min. in the dark.

For samples that are originally tested on either procedure A or B, any repeat testing or confirmation must be tested using the same procedure.

EIA Procedure A and B

1. Perform equipment maintenance and calibration, where necessary, as required by the manufacturer.

2. **Bring all of the reagents, except the Conjugate Concentrate, to room temperature** before beginning the assay procedure.

3. Prepare working concentrations of Wash Solution, Conjugate Solution, and Chromogen Solution. Mix gently, by inversion. Mix again just before use.

4. Remove strips not needed for the assay and replace them with labeled Null Strips, if necessary.

5. If sample identity is not maintained by an automatic procedure, identify the individual wells for each specimen or control on a data sheet.

6. **Add 200 µl of the controls or specimens to the appropriate wells** of the microwell plate. Two Positive Controls, two Low Positive Controls, and three Negative Controls should be assayed on each plate or partial plate of specimens.

7. Cover the microwell plate with a plate sealer or use other means to minimize evaporation.
   Procedure A: Incubate the plate for 60 to 65 minutes at 37±1°C using a dry-heat static incubator.
   Procedure B: Incubate the plate for 60 to 65 minutes at 37±1°C using a shaker incubator.

8. At the end of the incubation period, carefully remove the plate cover and aspirate the fluid from each well into a biohazard container. **Wash the microwell plate or strip a minimum of five times** with the Wash Solution (at least 400µl/well/wash). **Soak for 30 to 60 seconds between each wash.** Aspirate the Wash Solution after each wash. After the last wash, if excess liquid remains, blot the inverted plate on clean, absorbent paper towels. NOTE: Grasp the plate holder firmly at the center of the long sides before inverting to blot.

9. **Add 100 µl of Working Conjugate Solution to each well** containing a specimen or control.

10. Cover the microwell plate with a plate sealer or use other means to minimize evaporation. Incubate the plate for 60 to 65 minutes at 37±1°C using either a dry-heat static incubator or shaker incubator as was utilized in Step 7.

11. At the end of the incubation period, carefully remove the plate cover and aspirate

the fluid in each well into a biohazard container. **Wash the plates a minimum of five times** with Wash Solution (at least 400 µl/well/wash). **Soak for 30 to 60 seconds between each wash.** Aspirate the Wash Solution after each wash. After the last wash, if excess liquid remains, blot the inverted plate on a clean, absorbent paper towel. NOTE: Grasp the plate holder firmly at the center of the long sides before inverting to blot.

12. **Add 100 µl of the Working Chromogen Solution to each well containing a specimen or control**. Cover the microwell plate with a fresh plate sealer or use other means to minimize evaporation. **Incubate plates in the dark for 30 to 33 minutes at room temperature**. (For example, cover the plates with black plastic or place in a drawer.)

13. Carefully remove the plate cover and **add 100 µl of EIA Stopping Reagent to each well** to terminate the reaction. **Tap the plate gently, or use other means to assure complete mixing. Complete mixing is required for acceptable results.**

14. **Read absorbance within 30 minutes,** after adding the EIA Stopping Reagent, using the 450 nm filter with 615 nm to 630 nm as the reference. (Blank on air.) Ensure that all strips have been pressed firmly into place before reading.

Decontamination

Dispose of all specimens and materials used to perform the test as though they contain an infectious agent. Disposal should comply with all applicable waste disposal requirements.[6-10]

QUALITY CONTROL

Determine the mean absorbances for the Negative Controls, Positive Controls, and Low Positive Controls by dividing the summation of their absorbance values by the number of acceptable controls. One Negative Control may be discarded if it is outside of the acceptable validation range. No Positive Controls may be discarded.

Mean Absorbance of the Negative Controls (NCX)

Determine the NCX as shown in the example below:

Negative Control Sample Number	Absorbance	Total Absorbance	=	0.099	= 0.033(NCX)
1	0.032	3		3	
2	0.034				
3	0.033				
	0.099				

The individual absorbance values of the Negative Controls must be greater than 0.000 AU and less than or equal to 0.100 AU. One Negative Control absorbance value may be discarded if it is outside this range. The NCX may be calculated from the two remaining absorbance values.

Mean Absorbance of the Positive Controls (PCX)

Determine the PCX as shown in the example below:

Positive Control Sample Number	Absorbance	Total Absorbance	=	3.006	= 1.503(PCX)
1	1.418	2		2	
2	1.588				
	3.006				

The PCX must be greater than or equal to 1.000 AU, and each Positive Control absorbance value must be within the range of 0.65 to 1.35 times the PCX. No Positive Control absorbance value may be discarded.

Both Positive Control absorbance values above are within the range of 0.65 to 1.35 times the PCX as shown by the calculation below.

$$0.65 \times PCX = 0.65 \times 1.503 = 0.977$$
$$1.35 \times PCX = 1.35 \times 1.503 = 2.029$$

Therefore, the acceptable range is 0.977 to 2.029.

Mean Absorbance of the Low Positive Controls (LPCX)

Determine the LPCX as shown in the example below:

Low Pos. Control Sample Number	Absorbance	Total Absorbance	=	0.820	= 0.410(LPCX)
1	0.394	2		2	
2	0.426				
	0.820				

The LPCX must be positive (i.e. greater than or equal to the assay cutoff value).

Cutoff Value
Determine the cutoff value by adding the NCX to 0.070 as shown in the example below:

NCX = 0.033
Cutoff Value = 0.033 + 0.070 = 0.103

Validation
A run is valid if the following criteria are met:

- The absorbance values of the Negative Controls are greater than 0.000 AU and less than or equal to 0.100 AU. One Negative Control value may be discarded. If two or more Negative Controls are out of limit, the run must be repeated.
- The mean absorbance value of the Positive Controls (PCX) must be greater than or equal to 1.000 AU and the individual absorbance values must be within range of 0.65 to 1.35 times the PCX. No Positive Control values may be discarded.
- The mean absorbance of the Low Positive Controls must be positive (≥ assay cutoff). No Low Positive Control absorbance values may be discarded.

INTERPRETATION OF RESULTS

The presence or absence of HBsAg is determined by relating the absorbance value of the specimen to the cutoff values. The cutoff value is determined by addition of 0.070 to the mean absorbance value of the Negative Controls (NCX). An example of values obtained from an assay run and the interpretation are as follows:

Example:

Negative Control OD values	0.032 0.034 0.033	Individual Negative Control OD Values Negative Control mean	 Valid 0.033
Positive Control OD values	**1.418** **1.588**	Positive Control mean Positive Control acceptable range	1.503 0.977-2.029 valid
Cutoff Value = 0.033 + 0.070 =	0.103		
Low Positive Control OD values	**0.394** **0.426**	Low Positive Control mean	0.410 valid
Patient OD values	**1.910** **0.295** 0.011 **0.726** 0.100	Interpretation	Reactive Reactive Nonreactive Reactive Nonreactive

Specimens with absorbance values that are <0.000 must be repeated. Those with values greater than the upper linearity limits of the reader should be reported as reactive.

Specimens with absorbance values less than the cutoff value are considered nonreactive by the Genetic Systems™ HBsAg EIA 2.0 and may be considered negative for HBsAg. Further testing is not required.

Specimens with absorbance values greater than or equal to the cutoff value are considered initially reactive by the Genetic Systems™ HBsAg EIA 2.0. Initially reactive specimens should be retested in duplicate to validate the initial test results. If, after repeat testing, the absorbance values of both duplicate specimens are less than the cutoff value, the original specimen may be considered nonrepeatedly reactive and negative for HBsAg. Reasons for nonrepeatedly reactive specimens include:

- improper washing of microwell plates
- cross-contamination of nonreactive specimens with HBsAg from a high titered specimen
- contamination of the Chromogen Reagent solution by oxidizing agents (sodium hypochlorite, hydrogen peroxide, etc.)
- contamination of the Stopping Reagent

If, after repeat testing, the absorbance value of either of the duplicates is greater than or equal to the cutoff value, the specimen must be considered repeatedly reactive. If a confirmation is performed, repeatedly reactive specimens must be confirmed by the Genetic Systems™ HBsAg Confirmatory Assay 2.0, a licensed neutralization procedure utilizing human anti-HBs. The specimen can be considered positive for HBsAg only if the HBsAg can be neutralized by the confirmatory procedure.

LIMITATIONS OF THE PROCEDURE

1. The Genetic Systems™ HBsAg EIA 2.0 Procedure and the Interpretation of Results must be followed when testing serum, plasma, or cadaveric serum specimens for the presence of HBsAg. The user of this kit is advised to read the package insert carefully prior to conducting the test. In particular, the test procedure must be carefully followed for sample and reagent pipetting, plate washing and timing of the incubation steps.

2. A designation of reactive for HBsAg must not be based on a single reactive test result. Additional testing, such as confirmatory testing, is required to establish the specificity of any specimen reactive by the screening procedure.

3. False positive results can be expected with a kit of this nature. The proportion of reactives that are false will depend on the sensitivity and specificity of the test kit and upon the prevalence of antibodies to HBsAg in the population being screened.

4. False negative results can occur if the quantity of marker present in the sample is too low for the detection limits of the assay, or if the marker which is detected is not present during the stage of disease in which a sample is collected.

5. Failure to add specimen or reagent as instructed in the procedure could result in a falsely negative test. Repeat testing should be considered where there is clinical suspicion of infection or procedural error.

6. An absorbance value of less than 0.000 AU indicates a procedural or instrument error which should be evaluated. That result is invalid and that specimen must be re-run.

7. Factors that can affect the validity of results include failure to add the specimen to the well, inadequate washing of microplate wells, failure to follow stated incubation times and temperatures, addition of wrong reagents to wells, the presence of metals, or splashing of bleach into wells.

PERFORMANCE CHARACTERISTICS OF SERUM AND PLASMA TESTING

Reproducibility

Intra-assay and inter-assay reproducibility of Genetic Systems™ HBsAg EIA 2.0 were assessed for Procedure A (static mode) and Procedure B (shaker mode) using a nine member precision panel. Each member of the precision panel was tested four times on five different days on each of three lots of Genetic Systems™ HBsAg 2.0 at six sites. The mean, standard deviation, and coefficient of variation of the absorbance values are shown in Table 1A (Procedure A) and Table 1B (Procedure B) below:

Table 1A
Reproducibility of Genetic Systems™ HBsAg EIA 2.0 Procedure A (Static)

Intra-assay Reproducibility					Inter-assay Reproducibility				
Panel Member	N	Mean OD	SD[1]	%CV	Panel Member	N	Mean OD	SD[2]	%CV
1	360	1.305	0.092	7.0%	1	360	1.305	0.142	10.9%
2	360	0.390	0.033	8.5%	2	360	0.390	0.050	12.8%
3	360	0.217	0.015	6.9%	3	360	0.217	0.026	12.0%
4	359*	0.125	0.013	10.4%	4	359*	0.125	0.018	14.4%
5	360	1.606	0.069	4.3%	5	360	1.606	0.148	9.2%
6	360	0.612	0.041	6.7%	6	360	0.612	0.066	10.8%
7	359*	0.323	0.022	6.8%	7	359*	0.323	0.035	10.8%
8	360	0.165	0.012	7.3%	8	360	0.165	0.018	10.9%
9	358*	0.030	0.006	20.0%	9	358*	0.030	0.010	33.3%

Table 1B
Reproducibility of Genetic Systems™ HBsAg EIA 2.0 Procedure B (Shaker)

Intra-assay Reproducibility					Inter-assay Reproducibility				
Panel Member	N	Mean OD	SD[1]	%CV	Panel Member	N	Mean OD	SD[2]	%CV
1	359*	1.901	0.082	4.3%	1	359*	1.901	0.191	10.0%
2	360	0.783	0.057	7.3%	2	360	0.783	0.089	11.4%
3	359*	0.453	0.026	5.7%	3	359*	0.453	0.051	11.3%
4	360	0.255	0.018	7.1%	4	360	0.255	0.029	11.4%
5	360	2.069	0.061	2.9%	5	360	2.069	0.202	9.8%
6	360	1.109	0.059	5.3%	6	360	1.109	0.101	9.1%
7	360	0.645	0.037	5.7%	7	360	0.645	0.060	9.3%
8	360	0.330	0.022	6.7%	8	360	0.330	0.032	9.7%
9	355*	0.033	0.006	18.2%	9	355*	0.033	0.008	24.2%

*Outliers not included in statistical calculations.
[1]NCCLS Vol. 12 No.4, p.32, Eq 11. [2]NCCLS Vol. 12 No.4. p.33, Eq's 12 and 13.

Specificity

Reactivity in Random Donor Populations

In clinical investigations performed at five blood centers and a plasmapheresis center, 19,319 specimens from random blood donors were tested for HBsAg. The proportions of these specimens found initially and repeatedly reactive by Genetic Systems™ HBsAg EIA 2.0 for both Procedure A (static mode) and Procedure B (shaker mode) are shown in Table 2. The presence of HBsAg in repeatedly reactive specimens was confirmed by neutralization with human anti-HBs using the Genetic Systems™ HBsAg Confirmatory Assay 2.0.

Table 2
Detection of HBsAg in Serum from Blood Donors

Group	Total Tested	Procedure A (Static) Non-Reactive	Procedure A (Static) Initially Reactive	Procedure A (Static) Repeatedly Reactive	Procedure B (Shaker) Non-Reactive	Procedure B (Shaker) Initially Reactive	Procedure B (Shaker) Repeatedly Reactive	Confirmed Positive
Random Donors, Site A	2000	1993 (99.65%)	7 (0.35%)	2 (0.10%)	1991 (99.55%)	9 (0.45%)	2 (0.10%)	0
Random Donors, Site B	2000	1980 (99.00%)	20 (1.00%)	17 (0.85%)	1996 (99.80%)	4 (0.20%)	3 (0.15%)	0
Random Donors, Site E	4369	ND*	ND*	ND*	4353 (99.63%)	16 (0.37%)	3 (0.07%)	0
Random Donors, Site F	6914	ND*	ND*	ND*	6848 (99.05%)	66 (0.95%)	9 (0.13%)	0
Total Serum Donors	15,283	3973 (99.33%)	27 (0.67%)	19 (0.47%)	15,188 (99.38%)	95 (0.62%)	17 (0.11%)	0 (0.00%)

ND* = Not Done

Detection of HBsAg in Plasma from Blood Donors

Group	Total Tested	Procedure A (Static) Non-Reactive	Procedure A (Static) Initially Reactive	Procedure A (Static) Repeatedly Reactive	Procedure B (Shaker) Non-Reactive	Procedure B (Shaker) Initially Reactive	Procedure B (Shaker) Repeatedly Reactive	Confirmed Positive
Random Donors, Site C	1520	1504 (98.95%)	16 (1.05%)	15 (0.99%)	1498 (98.55%)	22 (1.45%)	20 (1.32%)	0
Random Donors, Site D	2516	2511 (99.80%)	5 (0.20%)	2 (0.08%)	2514 (99.92%)	2 (0.08%)	2 (0.08%)	1
Total Plasma Donors	4036	4015 (99.48%)	21 (0.52%)	17 (0.42%)	4012 (99.41%)	24 (0.59%)	22 (0.55%)	1 (0.02%)

Detection of HBsAg in Serum and Plasma from Blood Donors

Group	Total Tested	Procedure A (Static) Non-Reactive	Procedure A (Static) Initially Reactive	Procedure A (Static) Repeatedly Reactive	Procedure B (Shaker) Non-Reactive	Procedure B (Shaker) Initially Reactive	Procedure B (Shaker) Repeatedly Reactive	Confirmed Positive
Total Serum and Plasma Donors	19,319	7988 (99.40%)	48 (0.60%)	36 (0.45%)	19,200 (99.38%)	119 (0.62%)	39 (0.20%)	1 (0.01%)

Specificity of the Genetic Systems™ HBsAg EIA 2.0 was estimated from the results of screening tests in random U.S. blood and plasma donors. Specificity was estimated by the following formula:

$$\frac{(\#\text{ normal donor specimens} - \#\text{ repeatedly reactive specimens})}{(\#\text{ normal donor specimens} - \text{repeatedly reactive specimens confirmed positive for HBsAg})} \times 100$$

A total of 8036 donor specimens were tested with procedure A and 19,319 donor specimens were tested with procedure B of Genetic Systems™ HBsAg EIA 2.0; 36 of these specimens were repeatedly reactive by procedure A; 39 specimens were repeatedly reactive by procedure B; one (1) specimen was confirmed to be positive for HBsAg with procedures A and B. Thus the Genetic Systems™ HBsAg EIA 2.0 has an estimated specificity of 99.55% (95%; binomial confidence interval[11] = (0.9940, 0.9970)) for procedure A; and of 99.80% (95%; binomial confidence interval[11] = (0.9973, 0.9987)) for procedure B.

Sensitivity

The sensitivity of the Genetic Systems™ HBsAg EIA 2.0 was determined in three different product lots by testing dilutions of purified antigens (ad and ay) in human serum at three clinical sites. In Table 3, the mean absorbance to cutoff ratio for each antigen concentration (ng/mL) is presented for each incubation procedure (static and shaker) at each of the 3 sites.

Table 3: Detection of Purified HBsAg *ad* and *ay* subtypes

HBsAg Subtype	ng/ml	Replicates	Procedure A (Static) Mean Absorbance/cutoff Site 1	Site 2	Site 3	Procedure B (Shaker) Mean Absorbance/cutoff Site 1	Site 2	Site 3
ad	2.10	9	8.40	7.89	7.85	13.94	14.66	12.92
ad	1.10	9	4.31	4.19	4.04	8.35	8.95	7.68
ad	0.90	9	3.52	3.50	3.51	6.76	7.42	6.44
ad	0.70	9	2.71	2.75	2.69	5.50	6.24	5.23
ad	0.60	9	2.03	2.11	2.10	4.11	4.78	4.07
ad	0.50	9	1.79	1.77	1.73	3.61	4.00	3.45
ad	0.40	9	1.80	1.74	1.69	3.33	3.74	3.26
ad	0.30	9	1.39	1.43	1.37	2.61	3.01	2.62
ad	0.20	9	1.03	1.01	1.07	1.89	2.13	1.47
ad	0.10	9	0.49	0.45	0.53	0.85	0.91	0.81
ay	2.60	9	7.82	7.52	7.17	13.28	13.82	12.22
ay	1.30	9	4.08	4.01	3.91	7.88	8.61	7.26
ay	1.10	9	3.32	3.35	3.16	6.68	7.35	6.10
ay	0.90	9	2.78	2.70	2.69	5.77	6.18	5.32
ay	0.60	9	2.11	2.04	1.93	4.42	4.84	4.08
ay	0.50	9	1.82	1.80	1.91	3.82	4.17	3.53
ay	0.40	9	1.64	1.61	1.52	3.20	3.44	2.89
ay	0.30	9	1.24	1.23	1.25	2.54	2.98	2.30
ay	0.20	9	0.96	0.96	1.01	1.90	2.01	1.66
ay	0.10	9	0.56	0.55	0.63	1.06	1.11	0.97
Negative	0.00	9	0.29	0.24	0.34	0.39	0.32	0.34

Reactivity in Patients Diagnosed with Hepatitis B

One hundred (100) specimens from individuals diagnosed with acute hepatitis were tested with procedures A and B of the Genetic Systems™ HBsAg EIA 2.0. Of the 100 specimens, 94 were reactive with a licensed HBsAg EIA, all 94 specimens were reactive with Genetic Systems™ HBsAg EIA 2.0, procedure B; 93 specimens were reactive with Genetic Systems™ HBsAg EIA 2.0, procedure A.

Of 106 specimens from individuals diagnosed with chronic hepatitis B, all were reactive with Genetic Systems™ HBsAg EIA 2.0, procedures A and B, and in 100% agreement with a licensed HBsAg EIA.

Of 25 anti-Delta positive specimens, all were reactive with Genetic Systems™ HBsAg EIA 2.0, procedures A and B, and in 100% agreement with a licensed HBsAg EIA.

Reactivity in Seroconversion Panels

The sensitivity of Genetic Systems™ HBsAg EIA 2.0, procedures A and B, was assessed in 20 seroconversion panels purchased from Boston Biomedica, Inc. (West Bridgewater, MA) and 1 seroconversion panel purchased from Serologicals, Inc. (Clarkston, GA). The number of the bleed at which HBsAg was detected (S/CO ≥ 1.0) by the Genetic Systems™ HBsAg EIA 2.0, procedures A and B, is compared to other licensed HBsAg assays (as recorded in the certificates of analysis) in Table 4 below.

Table 4: Genetic Systems™ HBsAg EIA 2.0 Reactivity in HBsAg Seroconversion Panels

Panel I.D.#	Genetic Systems™ HBsAg EIA 2.0 Procedure A	Genetic Systems™ HBsAg EIA 2.0 Procedure B	HBsAg EIA #1 (overnight)	HBsAg EIA #2	HBsAg RIA (overnight)
901	1**	1**	1**	3	2
902	9	7**	9	10	10
903	4	3**	5	5	5
904	3	2**	3	3	3
905	1**	1**	1**	1**	1**
906	2**	2**	2**	2**	2**
907	6**	6**	6**	6**	6**
908	6	5**	7	7	7
909	4**	4**	4**	5	5
910	3**	3**	3**	3**	4
911	21	20**	21	23	23
912	8	7**	8	8	8
913	1**	1**	1**	2	2
914	3	2**	5	6	5
915	10	4**	9	12	11
917	3	2**	3	3	3
918	2**	2**	3	3	3
919	6	4**	6	5	5
920	3**	3**	3**	3**	3**
924	3	2**	3	4	4
405651	3	2**	3	NT	NT

*NT: Not Tested **Earliest detection

As can be seen from Table 4, the Genetic Systems™ HBsAg EIA 2.0 detected the presence of HBsAg as early as, or earlier than the licensed HBsAg assays in all 21 seroconversion panels using procedure B (shaker), and in 19 of the 21 seroconversion panels using procedure A (static).

PERFORMANCE CHARACTERISTICS OF CADAVERIC SPECIMEN TESTING

Reproducibility

Inter-assay reproducibility of Genetic Systems™ HBsAg EIA 2.0 was assessed for Procedure A (static mode) using twenty post-mortem sera and twenty normal donor sera, spiked with HBsAg positive serum to give reactivity near the cutoff. Each of the samples was tested once on six different days on each of three lots of Genetic Systems™ HBsAg EIA 2.0 at one site. For inter-assay reproducibility over all lots, percent coefficient of variation (%CV) ranged from 6.3% to 11.8% for the spiked post-mortem samples and from 5.9% to 12.6% for the spiked normal donor samples.

Specificity

In a clinical investigation at one site, sixty-three post-mortem samples and sixty-three normal donor samples were tested concurrently with procedure A (static mode) of Genetic Systems™ HBsAg EIA 2.0. The mean signal to cutoff (S/CO) ratio for 63 post-mortem samples was 0.347 and the mean S/CO ratio for the 63 normal donors was 0.185. The presence of HBsAg in repeatedly reactive specimens was confirmed by neutralization with human anti-HBs using procedure A (static mode) of Genetic Systems™ HBsAg Confirmatory Assay 2.0. Results are presented in Table 5 below.

Table 5
Reactivity with Genetic Systems™ HBsAg EIA 2.0 Procedure A

Population	Number Tested	Nonreactive	Initially Reactive	Repeatedly Reactive	Confirmed Positive
Post-mortem	63	62 (98.41%)	1 (1.59%)	1 (1.59%)	0 (0.0%)
Normal Donor	63	63 (100.0%)	0 (0.0%)	NA	NA

NA = Not Applicable

Specificity of Genetic Systems™ HBsAg EIA 2.0 (Procedure A) was estimated by the following formula:

$$\frac{(\#\text{ specimens} - \#\text{ repeatedly reactive specimens})}{(\#\text{ specimens} - \#\text{ repeatedly reactive specimens confirmed positive for HBsAg})} \times 100$$

A total of sixty-three post-mortem specimens were tested with procedure A of Genetic Systems™ HBsAg EIA 2.0; one (1.59%) of these specimens was repeatedly reactive but did not confirm positive for HBsAg. Thus, the Genetic Systems™ HBsAg

EIA 2.0 (Procedure A) has an estimated specificity of 98.4% [95%; binomial confidence interval[11] = (94.39%, 100%)] for post-mortem specimens.

Sensitivity

Sixty-six post-mortem samples and sixty-six normal donor samples were pre-screened for HBsAg and antibody to HBsAg and found to be nonreactive. Each sample was divided into two portions. One portion of each post-mortem and normal donor sample was spiked at a potency near cutoff with a positive serum containing HBsAg ad/ay and the remaining portion was left unspiked. The sixty-six spiked and unspiked post-mortem samples were tested concurrently with sixty-six spiked and unspiked normal donor specimens on the same run of Genetic Systems™ HBsAg EIA 2.0 (Procedure A). Spiked specimens were expected to be reactive and therefore were not retested in duplicate. The presence of HBsAg in initially reactive specimens was confirmed by neutralization with human anti-HBs using procedure A (static mode) of Genetic Systems™ HBsAg Confirmatory Assay 2.0. Results are presented in Table 6 below.

Table 6
Reactivity with Genetic Systems™ HBsAg EIA 2.0 Procedure A

Population	Number Tested	Non-reactive	Initially Reactive	Repeatedly Reactive	Confirmed Positive
Spiked Post-mortem	66	0 (0.00%)	66 (100.0%)	NT	66 (100.0%)
Unspiked Post-mortem	66	65 (98.5%)	1 (1.5%)	QNS	0 (0.0%)
Spiked Normal Donor	66	0 (0.00%)	66 (100.0%)	NT	66 (100.0%)
Unspiked Normal Donor	66	66 (100.0%)	0 (0.00%)	NA	NA

NT = Not Tested
NA = Not Applicable
QNS = Quantity Not Sufficient

As can be seen in Table 6, of sixty-six post-mortem samples and sixty-six normal donor samples, spiked at a potency near cutoff and tested concurrently, all (100.00%) were reactive with Genetic Systems™ HBsAg EIA 2.0 (Procedure A) and all confirmed positive with Genetic Systems™ HBsAg Confirmatory Assay 2.0 (Procedure A).

Furthermore, according to the Student's t - test, there is no significant statistical difference between the spiked post-mortem mean optical density signal and that of the spiked normal donor mean optical density signal (two sample assuming unequal variances). These results demonstrate that the detection of HBsAg in post-mortem samples is comparable to the detection in normal donors.

BIBLIOGRAPHY

1. Delmonico FL, Snydman DR: Organ donor screening for infectious diseases. **Transplantation** 65 (5): 603-610. 1998.

2. Zuckerman AJ: Viral hepatitis with special reference to hepatitis B. **Immunoassays for the 80's.** eds Voller A, Bartlett A, and Bidwell D, University Park Press. 361-373, 1981.

3. Wolters G, Kuijpers L, Kacaki J, Schuurs AHWM: Solid-phase enzyme-immunoassay for detection of hepatitis B surface antigen. **J Clin Pathol** 29(10):873-879. 1976.

4. David GS, Present W, Martinis J, Wang R, Bartholomew R, Desmond W, Sevier ED: Monoclonal antibodies in the detection of hepatitis infection. **Med Lab Sci** 38:341-348, 1981.

5. Kennedy RC, Ionescu-Matiu I, Adler-Storthz K, Henkel RD, Sanchez Y, Dreesman GR: Characterization of anti-hepatitis B surface antigen monoclonal antibodies. **Intervirology** 19:176-180, 1983.

6. Bos ES, van der Doelen AA, van Rooy N, Schuurs AHWM: 3,3',5,5'-tetramethylbenzidine as an ames test negative chromogen for horseradish peroxidase in enzyme immunoassay. **J Immunoassay** 2:187-204, 1981.

7. Garner RC, Walpole AL, Rose FL: Testing of some benzidine analogues for microsomal activation to bacterial mutagens. **Cancer Letters** 1:39-42, 1975.

8. Bond WW, Favero MS, Petersen NJ, Ebert JW: Inactivation of hepatitis B virus by intermediate-to-high-level disinfectant chemicals. **J Clin Microbiol** 18:535-538, 1983.

9. U.S. Environmental Protection Agency, Office of Solid Waste: **EPA Guide for Infectious Waste Management**, Washington D.C., 1987. (USG PO 530-SW-86-014).

10. Sehulster LM, Hollinger FB, Dreesman GR, Melnick JL: Immunological and biophysical alteration of hepatitis B virus antigens by sodium hypochlorite disinfection. **Appl and Envi Microbiol** 42:762-767, 1981.

11. Blythe CR, Still HA: Binomial confidence intervals. **J Amer Stat Assoc** 78:108-116, 1983.

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